510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE

A. 510(k) Number: K033743

B. Purpose for Submission:
Premaket Notification 510(k) of intention to manufacture and market the Cozart® EIA Amphetamines Oral Fluid Microplate Kit.

C. Analyte: Amphetamine

D. Type of Test: Qualitative competitive enzyme immunoassay for the detection of amphetamines in human oral fluid.

E. Applicant: Cozart Bioscience, Ltd**.**

F. Proprietary and Established Names:

Cozart® EIA Amphetamines Oral Fluid Microplate Kit

G. Regulatory Information:

1. Regulation section: 21CFR §862.3100 Amphetamine test system
2. Classification: Class II
3. Product Code: DKZ
4. Panel: Toxicology (91)

H. Intended Use:

1. Intended use(s):

The Cozart® EIA Amphetamines Oral Fluid Microplate Kit is intended for use in clinical and forensic laboratories when used in conjunction with the Cozart® RapiScan Oral Fluid collection system. Using this collection system it provides qualitative screening results for amphetamines in human oral fluid at a cutoff concentration of 15ng/mL. This is equal to 45ng/mL in neat oral fluid as the collection system involves a 1:3 dilution of the sample.

2. Indication(s) for use:

The Cozart® EIA Amphetamines Oral Fluid Microplate Kit is intended for use in clinical and forensic laboratories when used in conjunction with the Cozart® RapiScan Oral Fluid collection system. Using this collection system it provides qualitative screening

results for amphetamines in human oral fluid at a cutoff concentration of 15ng/mL. This is equal to 45ng/mL in neat oral fluid as the collection system involves a 1:3 dilution of the sample.

3. Special condition for use statement(s):

This assay is for professional use only and provides only a preliminary analytical result. Clinical consideration and professional judgement must be applied to any drug of abuse test result, particularly in evaluating a preliminary positive result. In order to obtain a more confirmed analytical result a more specific alternative chemical method is needed. Gas Chromatography/ Mass Spectrophotometry (GC/MS) is the preferred confirmatory method.

4. Special instrument Requirements:

Automated microtitre plate reader with a 450nm filter. Automated microtitre plate washing machine, manual microtiter plate washer or 350µL eight-channel pipette for dispensing diluted wash buffer.

I. Device Description:

The Cozart® EIA Amphetamine Oral Fluid Microplate Kit is supplied with the following reagents –
- a microtitre plate coated with antibody
- enzyme conjugate reagent
- wash buffer
- substrate solution
- stop solution
- four calibrators (0, 2, 15, and 50 ng/mL Amphetamine in synthetic oral fluid matrix)

Materials required but not provided –
- positive and negative controls
- automated microtitre plate reader with a 450nm filter
- precision pipettes with disposable tips.
- automated microtitre plate washing machine
- manual microtitre plate washer or 350µL eight-channel pipette for dispensing diluted wash buffer
- a timer for timing 30 minute intervals
- a clean measuring cylinder for dilution of wash buffer concentrate
- distilled or deionized water

J. Substantial Equivalence Information:

1. Predicate device name(s):

Amphetamine-Specific Intercept™ MICRO-PLATE EIA

2. Predicate K number(s): K992918

3. Comparison with predicate:

Parameter	Cozart® EIA Amphetamine Oral Fluid Microplate Kit	Amphetamine-Specific Intercept™ MICRO-PLATE EIA
Intended Use	Qualitative test for amphetamines in human oral fluid with a 45ng/mL cutoff. Recommended confirmation of positive results by GC/MS.	Qualitative test for amphetamines in human oral fluid with a 100ng/mL cutoff. Recommended confirmation of positive results by GC/MS.
Target Population	Clinical and forensic samples.	Clinical samples
Design	Competitive ELISA	Competitive ELISA
Enzyme	Horse Radish Peroxidase	Horse Radish Peroxidase
Results	Read spectrophotometrically at 450nm.	Read spectrophotometrically at 450nm.
Calibrators	0, 2, 15, 50ng/mL	0, 100ng/mL
Matrix	Human Oral Fluid	Human Oral Fluid
Controls	None supplied but Cozart recommends using external controls.	50, 200 ng/mL
Method Comparison	163 samples were tested, 67 screened positive for amphetamines, of which 62 were confirmed positive by GC/MS. 96 samples screened negative for amphetamines and 94 were confirmed negative by GC/MS. 96% Agreement as compared to GC/MS.	89% Agreement as compared to GC/MS.
Precision	CV (%) of 2.7 – 12.4%	CV (%) of 3.5 – 7.9%
Sensitivity	1.2 ng/mL	Unknown
Specificity	20 potential interferents tested – none cross-reacted.	47 potential interferents tested – none cross-reacted.

K. Standard/Guidance Document Referenced (if applicable):

Cut-Off validation Study was conducted with cutoff concentration of ± 50% of the value. Interference screening to identify exogenous and endogenous compounds was done in accordance with the NCCLS EP7-A.

L. Test Principle: The Cozart® EIA Amphetamine Oral Fluid Microplate Kit is a competitive enzyme immunoassay for the detection of amphetamines in human oral fluid. The wells of the microtitre strips are coated with anti-Amphetamine antibody. During the first incubation, the horseradish peroxidase (HRP) labelled amphetamine derivative competes with the free amphetamine in the patients sample for the anti-Amphetamine antibody binding sites on the microtitre strips. The wells are washed to remove any excess enzyme material prior to addition of the TMB substrate solution. Addition of the stop solution terminates the reaction and absorbances are read spectrophotometrically at 450nm.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
    a. Precision/Reproducibility:

Four calibrators (0, 2, 15, and 50ng/mL) and three samples (0, 7.7 and 22.5ng/mL) were tested in duplicate every day for twenty days. The samples were a negative, a negative sample fortified with amphetamine at 50% below the cutoff and a negative sample fortified with amphetamine at 50% above the cutoff. The within assay, within day, between assay, between day and total precision for the absorbance readings is displayed below.

Inter-Assay Precision

	Between Assay		
Sample	Mean	SD	CV%
Cal 1	2.287	0.140	6.11
Cal 2	1.406	0.099	7.02
Cal 3	0.848	0.069	8.11
Cal 4	0.575	0.063	10.91
Sample 1	2.252	0.159	7.05
Sample 2	1.090	0.104	9.55
Sample 3	0.771	0.075	9.73

Inter-Assay Precision

	Between Day		
Sample	Mean	SD	CV%
Cal 1	2.287	0.132	5.79
Cal 2	1.406	0.092	6.54
Cal 3	0.848	0.066	7.84
Cal 4	0.575	0.061	10.59
Sample 1	2.252	0.153	6.78
Sample 2	1.090	0.094	8.66
Sample 3	0.771	0.073	9.41

Intra-Assay Precision

Sample	Within Assay		
	Mean	SD	CV%
Cal 1	2.320	0.06	2.74
Cal 2	1.427	0.041	2.96
Cal 3	0.861	0.029	3.35
Cal 4	0.586	0.036	7.11
Low	2.286	0.063	2.85
Medium	1.109	0.042	3.62
High	0.784	0.03	3.94

Intra-Assay Precision

Sample	Within Day		
	Mean	SD	CV%
Cal 1	2.350	0.076	3.38
Cal 2	1.446	0.057	4.14
Cal 3	0.872	0.033	3.98
Cal 4	0.593	0.036	6.97
Low	2.315	0.077	3.47
Medium	1.122	0.065	5.81
High	0.794	0.036	4.75

Total Precision

Sample	Within Assay		
	Mean	SD	CV%
Cal 1	2.287	0.154	6.73
Cal 2	1.406	0.110	7.82
Cal 3	0.848	0.075	8.84
Cal 4	0.575	0.071	12.35
Low	2.252	0.173	7.68
Medium	1.090	0.118	10.83
High	0.771	0.081	10.54

b. Linearity/assay reportable range:

Not applicable. This test is for qualitative determinations.

c. Traceability (controls, calibrators, or method):

Each of the four cocaine calibrators supplied with the Cozart EIA Amphetamines Oral Fluid Microplate Kit were confirmed by GC/MS, the results are displayed below.

Calibrator Value (ng/mL)	GC/MS Cocaine Concentration (ng/mL)
0	0
2	1.67
15	14.67
50	50.33

The sponsor will make available to the user a calibrator (control) protocol through Cozart Technical Services. Information for technical assistance is provided in the package insert.

d. Detection limit:

The sensitivity was calculated by testing the 0 calibrator twenty times in a single assay. A calibration curve was plotted with the four calibrators. The mean absorbance of the 20 zeros minus two standard deviations was calculated. The sensitivity was calculated by reading this absorbance value off the calibration curve. The sensitivity of the Cozart EIA Amphetamines Oral Fluid Microplate EIA is 1.2ng/mL.

e. Analytical specificity:

Twenty potentially interfering unrelated substances were tested for cross reactivity in the Cozart Amphetamine Oral Fluid Kit and none were found to cross react. Eleven related compounds were tested and seven showed a level of cross reactivity. The substances were prepared in a negative oral fluid sample prior to testing in the assay. A calibration curve was plotted using the four calibrators to estimate the concentration of each sample.

Compound	**ng/mL Tested**	**Apparent Amphetamine ng/mL**	**% Cross Reactivity**
MDA	5	10.8	216
	10	18.8	188
MAMP	1,000	9.1	0.91
	5,000	86.6	1.73
MBDB	5,000	5.2	0.10
	10,000	19.9	0.20
MDMA	1,000	10.0	1.00
	5,000	90.0	1.80
MDEA	5,000	7.7	0.15
	10,000	22.3	0.22
Fenfluramine	100,000	6.2	0.01
Tyramine	1,000	3.1	0.31
	5,000	42.9	0.86
(+) Ephedrine	10,000	0.9	0.009
	100,000	26.4	0.026
(-) Ephedrine	100,000	2.8	0.003
(+) Pseudo Ephedrine	100,000	10.3	0.010
(-) Pseudo Ephedrine	100,000	<2	<0.002
Morphine	100,000	<2	<0.002
Temazepam	100,000	<2	<0.002
Cocaine	100,000	<2	<0.002
Chloroquine	100,000	<2	<0.002
Diazepam	100,000	<2	<0.002
Amitriptyline HCL	100,000	<2	<0.002
Dextromethorphan	100,000	<2	<0.002
Ranitidine	100,000	6.6	0.007
Ascorbic acid	100,000	<2	<0.002
DL-Propranolol	100,000	<2	<0.002
Caffeine	100,000	<2	<0.002
Acetylsalicylic acid (Aspirin)	100,000	<2	<0.002
Acetaminophen (Paracetamol)	100,000	<2	<0.002
Quinalbarbitone	100,000	<2	<0.002
Methylphenidate	100,000	<2	<0.002
Buprenorphine	10,000	<2	<0.002
Phenobarbital	100,000	<2	<0.002
Nicotine	100,000	<2	<0.002
LSD	100,000	<2	<0.002
Cotinine	100,000	<2	<0.002

f. Assay cut-off:

Testing samples at the cutoff concentration, 50% above and 50% below was carried out to validate the cutoff concentration. The 50ng/mL calibrator was diluted with a negative oral fluid sample to 7.5, 15, and 22.5ng/mL. Ten replicates of each concentration were tested along with the four calibrators. The mean absorbance of the ten replicates of the 15ng/mL calibrator was used as the cutoff. The absorbances obtained for the 7.5ng/mL calibrator were all higher than the 15ng/mL cutoff calibrator. Similarly the absorbances obtained for the 22.5ng/mL calibrator were all lower than the 15ng/mL cutoff calibrator.

Sample Concentration	**7.5ng/mL**
Mean Absorbance	0.857
SD	0.04
CV (%)	4.51

Sample Concentration	**15ng/mL**
Mean Absorbance	0.723
SD	0.03
CV (%)	4.74

Sample Concentration	**22.5ng/mL**
Mean Absorbance	0.654
SD	0.02
CV (%)	3.29

2. Comparison studies:

a. Method comparison with predicate device:

163 samples were tested through the Cozart® EIA Amphetamine Oral Fluid Microplate Kit. 67 samples screened positive for amphetamines, these consisted of 62 samples from drug users attending drug dependency units. And 5 samples spiked at 60ng/mL to give samples around the cutoff. 62 of the samples that screened positive for amphetamines were then confirmed positive y GC/MS. 3 of the unconfirmed positive samples were confirmed positive for MDMA at >180ng/mL and 149ng/mL but negative for MDA and amphetamine and therefore classed as negative for the reason stated above. However they screened positive due to the small cross reactivity of the kit to MDMA. 96 samples screened negative and 94 were confirmed negative by GC/MS. Of the 163 samples tested 17 were between -50% cutoff and +50% cutoff.

New Device		GC/MS Negs	GC/MS Negs between -50% Cutoff and Cutoff	GC/MS between +50% Cutoff and Cutoff	Total GC/MS Pos	Percent Agreement with GC/MS
Pos	67	5	0	8	62	93
Neg	96	94	9	0	2	98

96% overall agreement as compared with GC/MS

b. Matrix comparison:

Not applicable. This device is indicated only for oral fluid specimens.

3. Clinical studies:

a. Clinical sensitivity:

Not applicable. Clinical studies are not typically submitted for this device type.

b. Clinical specificity:

Not applicable. Clinical studies are not typically submitted for this device type.

c. Other clinical supportive data (when a and b are not applicable):

3. Clinical cut-off:

Analytical characterization of performance around the cut-off was demonstrated in the precision studies.

5. Expected values/Reference range:

Not applicable.

N. Conclusion:

The submitted material in this premarket notification is complete and supports a substantial equivalence decision.